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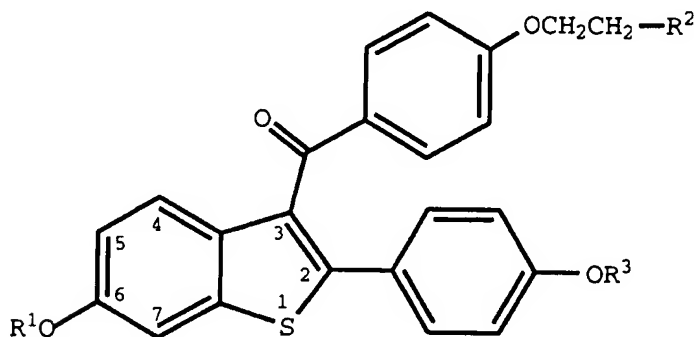
(71) Applicant : **ELI LILLY AND COMPANY**
Lilly Corporate Center
Indianapolis Indiana 46285 (US)

(72) Inventor : **Zuckerman, Steven Harold**
7710 Wawasee Drive
Indianapolis, Indiana 46250 (US)

(74) Representative : **Tapping, Kenneth George et al**
Lilly Industries Limited
European Patent Operations
Erl Wood Manor
Windsor Surrey GU20 6PH (GB)

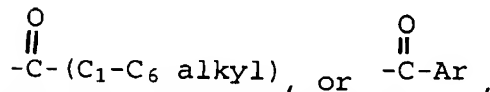
(54) **2-Phenyl-3-azoylbenzothiophenes for increasing macrophage function.**

(57) A method of increasing macrophage function comprising administering to a human in need thereof an effective amount of a compound having the formula



(I)

wherein R¹ and R³ are independently hydrogen, -CH₃,



wherein Ar is optionally substituted phenyl ;

R² is selected from the group consisting of pyrrolidine, hexamethyleneamino, and piperidino ; or a pharmaceutically acceptable salt of solvate thereof.

Also encompassed by the invention is a method of treating immunocompromised individuals comprising administering a compound of formula 1.

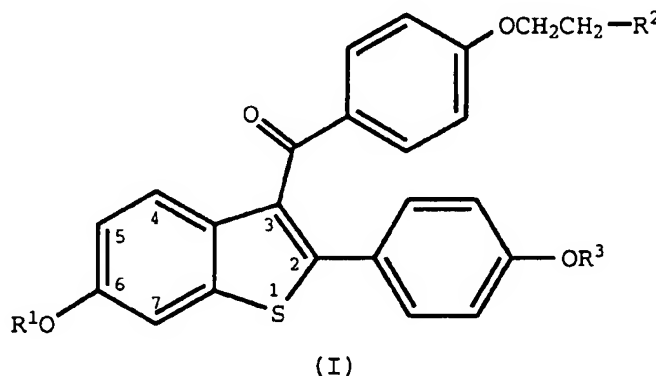
Macrophages play a central role in host defense through a variety of effector mechanisms involving both membrane related and secretory events (Gordon *et al.*, *Curr. Opin. Immunol.*, **4**, 25, 1992; Fuller, *Brit. Med. J.*, **48**, 65, 1992). Phagocytosis, chemotaxis and antigen presentation are membrane related processes involved in immunologic defense mechanisms necessary for host survival. The importance of macrophages in defense against microbes, immune surveillance, destruction of tumor cells, and in the clearing of senescent erythrocytes has been documented in man and in animal models characterized by the selective elimination of macrophages (Claassen *et al.*, *J. Immunol Meth.*, **134**, 153, 1990). Macrophages also contribute to host defense through secretion of bacteriostatic and bactericidal proteins, cytokines and lipid mediators, as well as oxygen and nitrogen reactive intermediates. The secretory capacity of the macrophage is central to its function as these cells secrete over 100 distinct mediators and are located in every organ (Nathan, *J. Clin. Invest.*, **79**, 319, 1987).

While aberrant activation of macrophage functions is associated with autoimmune diseases as well as both chronic and acute inflammatory processes, the reciprocal condition, suppression of macrophage effector functions, is associated with reoccurring infections of both opportunistic and non-opportunistic pathogens and contributes to increased morbidity and mortality. Populations associated with an immunocompromised state include burn patients, transplants, HIV infected individuals, cancer patients undergoing chemotherapy and surgical patients, notably those with a higher risk of infection as observed in thoracoabdominal patients.

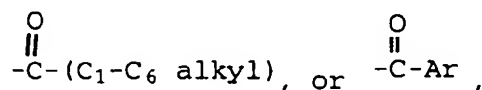
Current therapeutic approaches to these patients includes the use of intravenous infusion of macrophage derived cytokines notably the colony stimulating factors G-CSF, GM-CSF, and M-CSF (Nemunaitis, *Transfusion* **33**: 70, 1993). Supportive therapy with antibiotics and fluids is also used however the limitations of these approaches are demonstrated by the continued problems of infection in immunocompromised patients and the emergence of more deadly strains of antibiotic resistant organisms. Furthermore, infections of immunocompromised patients with opportunistic pathogens including *Pneumocystis* and *Cryptococcal* infections remain significant and result in complications despite various antibiotic protocols. Clearly, novel therapeutics which can selectively enhance macrophage effector functions to augment host defense would play a central role in the clinical management of these patients.

Estrogen has been reported to increase select macrophage effector functions including Fc mediated phagocytosis, class II antigen expression, and IL-1 secretion. These observations coupled with the known propensity of women to be more resistant to a variety of infections (Ahmed *et al.*, *Am. J. Path.*, **121**, 531, 1985) suggests that estrogen-like compounds may enhance macrophage effector functions and thus be beneficial in disease states associated with depressed host defense.

This invention provides methods for increasing macrophage function comprising administering to a human in need thereof an effective amount of a compound of formula I



wherein R¹ and R³ are independently hydrogen, -CH₃,



wherein Ar is optionally substituted phenyl;

R² is selected from the group consisting of pyrrolidino, hexamethylenimino, and piperidino; and pharmaceutically acceptable salts and solvates thereof.

Also encompassed by the invention is a method of treating an immunocompromised human comprising administering a compound of formula I to said human.

The current invention concerns the discovery that a select group of 2-phenyl-3-arylbenzothiophenes (benzothiophenes), those of formula I, are useful for increasing macrophage function. It is believed the benzothiophenes disclosed increase macrophage function, including class II antigen expression, (hence antigen presentation), Fc mediated phagocytosis, and/or cytokine release. The therapeutic and prophylactic treatments provided by this invention are practiced by administering to a human in need thereof a dose of a compound of formula I or a pharmaceutically acceptable salt or solvate thereof, that is effective to increase macrophage function.

The term "increasing macrophage function" is defined to include enhancement or augmentation of macrophage function or activation rate so as to augment a human's defense.

The compound of formula 1 should be useful in the treatment, both prophylactic and therapeutic, in immunocompromised persons, and in particularly in thoracoabdominal surgical infections, myeloid depressed patients following chemotherapy, burn patients, HIV infected individuals, and transplant patients undergoing immunosuppressive therapy. Additional uses would include prophylactic and therapeutic uses for reoccurring bacterial, protozoan, and fungal infections, as well as in patients with Myelodysplastic syndrome and aplastic anemia in which myeloid cells are largely non-functional. It is anticipated that in any clinical entity in which Colony Stimulating Factors are being used, the compounds of formula 1 would also be useful.

Raloxifene is a preferred compound of this invention and it is the hydrochloride salt of a compound of formula 1 wherein R¹ and R³ are hydrogen and R² is 1-piperidinyl.

Generally, at least one compound of formula I is formulated with common excipients, diluents or carriers, and compressed into tablets, or formulated as elixirs or solutions for convenient oral administration, or administered by the intramuscular or intravenous routes. The compounds can be administered transdermally, and may be formulated as sustained release dosage forms and the like.

The compounds used in the methods of the current invention can be made according to established procedures, such as those detailed in U.S. Patent Nos. 4,133,814, 4,418,068, and 4,380,635 all of which are incorporated by reference herein. In general, the process starts with a benzo[b]thiophene having a 6-hydroxyl group and a 2-(4-hydroxyphenyl) group. The starting compound is protected, acylated, and deprotected to form the formula I compounds. Examples of the preparation of such compounds are provided in the U.S. patents discussed above. The term "optionally substituted phenyl" includes phenyl and phenyl substituted once or twice with C₁-C₈ alkyl, C₁-C₄ alkoxy, hydroxy, nitro, chloro, fluoro, or tri(chloro or fluoro)methyl.

The compounds used in the methods of this invention form pharmaceutically acceptable acid and base addition salts with a wide variety of organic and inorganic acids and bases and include the physiologically acceptable salts which are often used in pharmaceutical chemistry. Such salts are also part of this invention. Typical inorganic acids used to form such salts include hydrochloric, hydrobromic, hydroiodic, nitric, sulfuric, phosphoric, hypophosphoric and the like. Salts derived from organic acids, such as aliphatic mono and dicarboxylic acids, phenyl substituted alkanolic acids, hydroxyalkanoic and hydroxyalkandioic acids, aromatic acids, aliphatic and aromatic sulfonic acids, may also be used. Such pharmaceutically acceptable salts thus include acetate, phenylacetate, trifluoroacetate, acrylate, ascorbate, benzoate, chlorobenzoate, dinitrobenzoate, hydroxybenzoate, methoxybenzoate, methylbenzoate, o-acetoxybenzoate, naphthalene-2-benzoate, bromide, isobutyrate, phenylbutyrate, β -hydroxybutyrate, butyne-1,4-dioate, hexyne-1,4-dioate, caprate, caprylate, chloride, cinnamate, citrate, formate, fumarate, glycollate, heptanoate, hippurate, lactate, malate, maleate, hydroxymaleate, malonate, mandelate, mesylate, nicotinate, isonicotinate, nitrate, oxalate, phthalate, teraphthalate, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, propionate, propionate, phenylpropionate, salicylate, sebacate, succinate, suberate, sulfate, bisulfate, pyrosulfate, sulfite, bisulfite, sulfonate, benzene-sulfonate, p-bromophenylsulfonate, chlorobenzenesulfonate, ethanesulfonate, 2-hydroxyethanesulfonate, methanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, p-toluenesulfonate, xylenesulfonate, tartarate, and the like. A preferred salt is the hydrochloride salt.

The pharmaceutically acceptable acid addition salts are typically formed by reacting a compound of formula I with an equimolar or excess amount of acid. The reactants are generally combined in a mutual solvent such as diethyl ether or benzene. The salt normally precipitates out of solution within about one hour to 10 days and can be isolated by filtration or the solvent can be stripped off by conventional means.

Bases commonly used for formation of salts include ammonium hydroxide and alkali and alkaline earth metal hydroxides, carbonates, as well as aliphatic and primary, secondary and tertiary amines, aliphatic diamines. Bases especially useful in the preparation of addition salts include ammonium hydroxide, potassium carbonate, methylamine, diethylamine, ethylene diamine and cyclohexylamine.

The pharmaceutically acceptable salts generally have enhanced solubility characteristics compared to the compound from which they are derived, and thus are often more amenable to formulation as liquids or emulsions.

Pharmaceutical formulations can be prepared by procedures known in the art. For example, the compounds can be formulated with common excipients, diluents, or carriers, and formed into tablets, capsules, suspensions, powders, and the like. Examples of excipients, diluents, and carriers that are suitable for such formulations include the following: fillers and extenders such as starch, sugars, mannitol, and silicic derivatives; binding agents such as carboxymethyl cellulose and other cellulose derivatives, alginates, gelatin, and polyvinyl pyrrolidone; moisturizing agents such as glycerol; disintegrating agents such as calcium carbonate and sodium bicarbonate; agents for retarding dissolution such as paraffin; resorption accelerators such as quaternary ammonium compounds; surface active agents such as cetyl alcohol, glycerol monostearate; adsorptive carriers such as kaolin and bentonite; and lubricants such as talc, calcium and magnesium stearate, and solid polyethyl glycols.

The compounds can also be formulated as elixirs or solutions for convenient oral administration or as solutions appropriate for parenteral administration, for instance by intramuscular, subcutaneous or intravenous routes. Additionally, the compounds are well suited to formulation as sustained release dosage forms and the like. The formulations can be so constituted that they release the active ingredient only or preferably in a particular part of the intestinal tract, possibly over a period of time. The coatings, envelopes, and protective matrices may be made, for example, from polymeric substances or waxes.

The particular dosage of a compound of formula I required to increase macrophage function or treat an immunocompromised individual, according to this invention, will depend upon the severity of the condition, the route of administration, and related factors that will be decided by the attending physician. Generally, accepted and effective daily doses will be from about 0.1 to about 1000 mg/day, and more typically from about 50 to about 200 mg/day. Such dosages will be administered to a subject in need thereof from once to about three times each day, or more often as needed to effectively treat or prevent the disease(s) or symptom(s).

It is usually preferred to administer a compound of formula I in the form of an acid addition salt, as is customary in the administration of pharmaceuticals bearing a basic group, such as the piperidino ring. It is preferred to administer a compound of the invention to a female, and further to an aging human (e.g. a postmenopausal female). For such purposes the following oral dosage forms are available.

Formulations

In the formulations which follow, "Active ingredient" means a compound of formula I.

Formulation 1: Gelatin Capsules

Hard gelatin capsules are prepared using the following:

Ingredient	Quantity (mg/capsule)
Active ingredient	0.1 - 1000
Starch, NF	0 - 650
Starch flowable powder	0 - 650
Silicone fluid 350 centistokes	0 - 15

The ingredients are blended, passed through a No. 45 mesh U.S. sieve, and filled into hard gelatin capsules.

Examples of specific capsule formulations of raloxifene that have been made include those shown below:

Formulation 2: Raloxifene capsule

	Ingredient	Quantity (mg/capsule)
5	Raloxifene	1
	Starch, NF	112
	Starch flowable powder	225.3
10	Silicone fluid 350 centistokes	1.7

Formulation 3: Raloxifene capsule

	Ingredient	Quantity (mg/capsule)
15	Raloxifene	5
	Starch, NF	108
20	Starch flowable powder	225.3
	Silicone fluid 350 centistokes	1.7

Formulation 4: Raloxifene capsule

	Ingredient	Quantity (mg/capsule)
30	Raloxifene	10
	Starch, NF	103
	Starch flowable powder	225.3
35	Silicone fluid 350 centistokes	1.7

Formulation 5: Raloxifene capsule

	Ingredient	Quantity (mg/capsule)
40	Raloxifene	50
	Starch, NF	150
45	Starch flowable powder	397
	Silicone fluid 350 centistokes	3.0

50 The specific formulations above may be changed in compliance with the reasonable variations provided.
A tablet formulation is prepared using the ingredients below:

55

Formulation 6: Tablets

	Ingr dient	Quantity (mg/tablet)
5	Active ingr di nt	0.1 - 1000
	Cellulose, microcrystalline	0 - 650
	Silicon dioxide, fumed	0 - 650
10	Stearate acid	0 - 15

The components are blended and compressed to form tablets.

Alternatively, tablets each containing 0.1 - 1000 mg of Active ingredient are made up as follows:

15 **Formulation 7: Tablets**

	Ingredient	Quantity (mg/tablet)
20	Active ingredient	0.1 - 1000
	Starch	45
	Cellulose, microcrystalline	35
25	Polyvinylpyrrolidone (as 10% solution in water)	4
	Sodium carboxymethyl cellulose	4.5
	Magnesium stearate	0.5
30	Talc	1

The active ingredient, starch, and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant powders which are then passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50°-60° C and passed through a No. 18 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate, and talc, previously passed through a No. 60 U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets.

Suspensions each containing 0.1 - 1000 mg of Active ingredient per 5 mL dose are made as follows:

40 **Formulation 8: Suspensions**

	Ingredient	Quantity (mg/5 ml)
45	Active ingredient	0.1 - 1000 mg
	Sodium carboxymethyl cellulose	50 mg
	Syrup	1.25 mg
	Benzoic acid solution	0.10 mL
50	Flavor	q.v.
	Color	q.v.
55	Purified water to	5 mL

Th active ingredi nt is passed through a No. 45 mesh U.S. sieve and mixed with the sodium carboxymethyl cellulose and syrup to form a smooth paste. The benzoic acid solution, flavor, and color are diluted with some of the water and added, with stirring. Sufficient water is then added to produce the required volume.

ASSAYSAssay 1

5 The procedure as set out in Freidman et al., *J. Clin. Invest.*, 75, 162-167 (1985) (herein incorporated by reference) is carried out, with certain modifications. Between five and one hundred mice are administered oral doses in the range of 1-10 mg/kg of a compound of formula 1 on a daily basis. Following the administration, macrophages are harvested and changes in both immune (Fc mediated) and non-immune phagocytosis are quantitated by using fluorescein conjugated yeast particles prepared based on Ragsdale, *J Immunol Meth*, 123:259, (1989). For immune mediated phagocytosis, fluorescein conjugated yeast is preincubated with mouse sera to promote opsonization. Increase in fluorescence uptake by macrophages is quantitated by an increase in fluorescent emission using excitation and emission wavelengths of 482 and 520 nm, respectively. This procedure is used with ex vivo or in vitro macrophage cultures and changes in fluorescence units quantitated.

An increase in fluorescent units, as compared to control indicates activity of compounds of formula 1.

Assay 2

20 The procedure as set out in Zuckerman et al., *Cell Immunol*, 103:207, (1986); *J Immunol*, 140:978 (1988) (herein incorporated by reference) is carried out. The ability to induce class II antigens and consequently promote antigen presentation is determined on ex vivo primary peritoneal macrophages and in vitro with the murine macrophage cell line P388D1. Between five and one hundred mice are dosed with a compound of formula 1 macrophages are harvested and probed with antibodies against class II antigens of the D haplotype. Increased class II antigen expression is determined by flow cytometry using the appropriate secondary antibodies. In vitro studies evaluate the effects of the compounds in increasing the basal level and gamma interferon inducible expression of class II antigen by flow cytometry. An increase in class II expression reflect an increase in macrophage activation.

Assay 3

30 The procedure as set out in Seow et al., *J. Immunol. Meth.*, 98, 113 (1987) (herein incorporated by reference) is carried out. The assay is used to evaluate increases in macrophage effector functions which uses measurements of 2-deoxyglucose uptake. Macrophages ex vivo and in vivo are plated in 96 well plates at 10^5 cells per well and incubated in phosphate buffered saline in the presence of 0.78 μ Ci/ml of 3H-deoxyglucose, and a compound of formula 1 is placed in the wells. Reduction in the amount of extracellular glucose reflects the uptake of this non-metabolizable glucose analog and consequently provides an independent assay for the determination of the state of macrophage activation mediated by the compound of formula 1. Increase in deoxyglucose uptake by the compound demonstrates the ability of the compounds to increase the state of macrophage activation.

Assay 4

40 The procedure as set out in Zuckerman, *Circ Shock*, 29, 279 (1989) (herein incorporated by reference) is carried out to illustrate the ability of the compounds of formula 1 to protect in murine sepsis and endotoxin lethality models. Between five and one hundred mice are dosed orally with 1-10 mg/kg with a compound of formula 1 for 1 week prior to sepsis challenge. Challenge is performed using a bolus IV endotoxin injection under condition in which an LD100 is achieved (200 μ g lipopolysaccharide). Exogenous glucocorticoids such as dexamethasone at 20 mg/kg serve as a positive control in increasing survival. The effects of the compound of formula 1 is also determined using a sepsis model involving cecal ligation and puncture. Sepsis by both Gram positive and Gram negative organisms results in an LD100 by 48 hours despite the use of antibiotics. An increase in the number of surviving animals or in survival time, as compared to control, demonstrates the activity of the compounds.

Assay 5

55 The ability of the compounds of formula 1 to increase the secretion of cytokines such as TNF is quantitated in vivo by serum measurements using commercially available TNF ELISAs specific for mouse TNF. Between five and one hundred mice are orally dosed with 1-10 mg/kg of a compound of formula 1 for one week prior to injection of a lethal or sublethal dose of lipopolysaccharide (200 and 1 μ g, respectively). At one hour post LPS

injection the mice are bled and the basal and LPS inducible amounts of serum TNF determined. Routinely, TNF levels below 10 pg/ml are observed prior to LPS injection and achieve levels of 5-20 ng/ml following LPS. The ability of the compounds to modulate the basal or inducible levels of TNF is determined. An increase in basal TNF without triggering massive systemic TNF release in compound treated mice demonstrates the activity of the compounds in promoting cytokine secretion. Finally, ex vivo and in vitro measurements of TNF release from peritoneal macrophages exposed to 1-5 μ M of a compound in vitro is also performed by ELISA to determine the extent of cytokine increase mediated by a compound of formula 1.

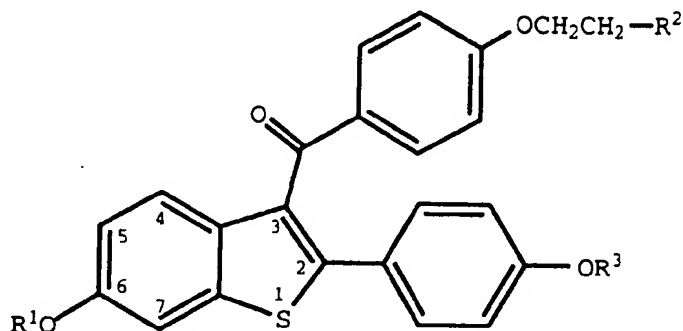
Assay 6

Five to fifty women are selected for the clinical study. The women are immunosuppressed. Because of the idiosyncratic and subjective nature of these disorders, the study has a placebo control group, i.e., the women are divided into two groups, one of which receives a compound of formula 1 as the active agent and the other receives a placebo. Women in the test group receive between 50-200 mg of the drug per day. They continue this therapy for 3-12 months. Accurate records are kept as to the number and severity of the symptoms in both groups and at the end of the study these results are compared. The results are compared both between members of each group and also the results for each patient are compared to the symptoms reported by each patient before the study began.

Utility of the compounds of formula I is illustrated by the positive impact they have in at least one of the assays described above.

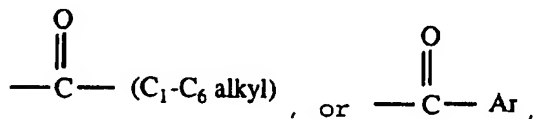
Claims

1. The use of a compound having the formula



(I)

wherein R¹ and R³ are independently hydrogen, -CH₃,



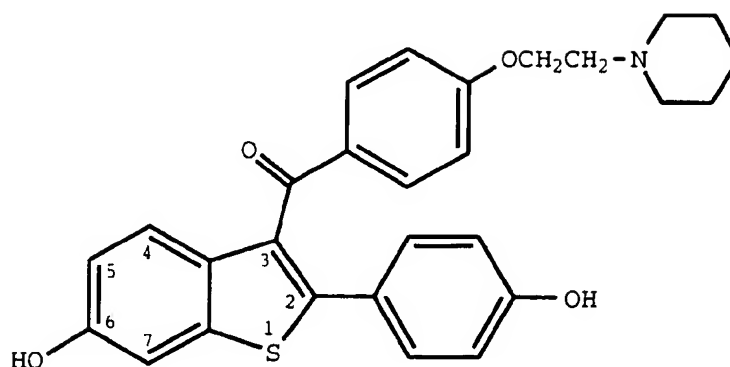
wherein Ar is optionally substituted phenyl;

R² is selected from the group consisting of pyrrolidino and piperidino; or a pharmaceutically acceptable salt or solvate thereof, in the preparation of a medicament useful for increasing macrophage function.

2. The use of Claim 1 wherein said compound is the hydrochloride salt thereof.

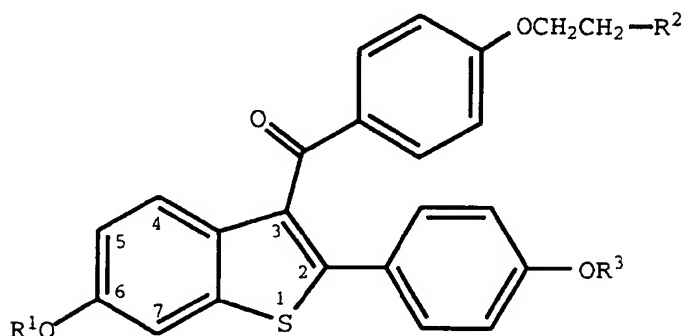
3. The use of Claim 1 wherein said medicament is prophylactic.

4. The use of Claim 1 wherein said compound is



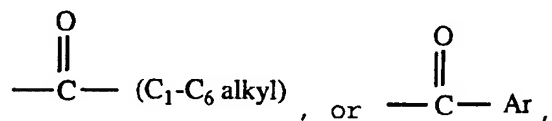
or its hydrochloride salt.

5. The use of a compound having the formula



(I)

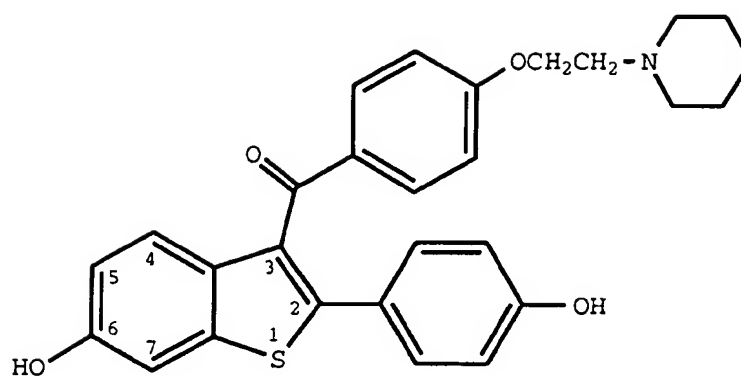
wherein R¹ and R³ are independently hydrogen, -CH₃,



wherein Ar is optionally substituted phenyl;

R² is selected from the group consisting of pyrrolidino and piperidino; or a pharmaceutically acceptable salt or solvate thereof, in the preparation of a medicament useful for treating an immunocompromised human.

6. The use of Claim 5 wherein said compound is



or its hydrochloride salt.



European Patent
Office

EUROPEAN SEARCH REPORT

Application Number
EP 94 30 9483

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)
A	US-A-5 075 321 (SCHREIBER) 24 December 1991 * the whole document * ---	1-6	A61K31/445 A61K31/40 A61K31/38
A	TRANS. ASSOC. AM. PHYSICIANS, vol. 100, 1987 pages 268-275, M.C. SANDERS ET AL. 'HORMONAL MODULATION OF MACROPHAGE CLEARANCE OF IgG-SENSITIZED CELLS' * the whole document * ---	1-6	
A	EP-A-0 222 385 (RESEARCH DEVELOPMENT CORPORATION OF JAPAN) 20 May 1987 * the whole document * ---	1-6	
A	BAILLIERE'S CLINICAL RHEUMATOLOGY, vol. 4, no.1, 1990 pages 13-31, S.A. AHMED ET AL. 'SEX HORMONES AND THE IMMUNE SYSTEM' * summary pages 26-27 * * page 25 * ---	1-6	TECHNICAL FIELDS SEARCHED (Int.Cl.6) A61K
D,A	US-A-4 418 068 (JONES) 29 November 1983 * abstract; claims * ---	1-6	
D,A	US-A-4 133 814 (JONES ET AL.) 9 January 1979 * abstract; claims * -----	1-6	
The present search report has been drawn up for all claims			
Place of search THE HAGUE		Date of completion of the search 23 March 1995	Examiner Hoff, P
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons A : member of the same patent family, corresponding document			

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